DISPATCH

Animal Evolution: Only Rocks Can Set the Clock

Molecular clocks have become the method of choice to date the tree of life. A new study demonstrates that there are limits to their precision, which may only be overcome by improving our knowledge of the fossil record.

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When did animals (the Metazoa) evolve [1-4]? Can we correlate the evolutionary history of animals with specific events in Earth history to understand what drove their origin and subsequent diversification [1-4]? For example, was the origin of animals triggered by the emergence of modern, well-oxygenated oceans [5], or did oxygenated oceans emerge as a consequence of the evolution of sponge-grade animals capable of removing dissolved carbon from the water column and sequestering it within sediments [6-7]? These and other similar questions have fascinated scientists for generations, and are united by the requirement of an accurate and precise timescale of animal evolution to answer them. Attempts to offer such a timescale often utilise methods based on the molecular clock. The molecular clock — first proposed by Emile Zuckerkandl and Linus Pauling in 1962 — works on the premise that the number of mutations independently accumulating in the genomes of living organisms is to some level proportional to the time that has elapsed since they shared a common ancestor [8]. However, a new study by dos Reis and colleagues [1] in this issue of *Current Biology* demonstrates that, at present, these methods are not precise enough to correlate milestones in early animal history with events in the geological record.

Scientists have long relied on fossils to draft a timescale of animal evolution, but the fossil record is beset by several rather annoying flaws. Due to the vagaries of sedimentary, tectonic and erosive processes, the fossil record is incomplete [9]. Furthermore, the oldest known fossil of a given species identifies only its first appearance in the rock record, which generally corresponds to a time when the species was already well established, with stable and abundant populations. The biological origin of a given species will always be older than its first

appearance in the fossil record [10]. Finally, the deeper we delve into Earth history, the harder it becomes to recognise specific fossils as the extinct relatives of living species. DNA and proteins do not preserve well, and the genealogical relationships of fossils close to the root of the animal tree can only be defined by the presence of shared morphological features (homologies). Yet, the further we move back in time, the smaller the number of homologies becomes [2]. As a result, whereas we can intuitively visualise the appearance of the last common ancestor of, say, humans and chimps, an animal that lived ~6.5–10 million years ago [10], picturing the last common ancestor of humans and sea cucumbers (an animal that inhabited the Earth's oceans at least ~515.5 million years ago [1-4,10]) is rather more speculative. Even experts, when faced with the fossil remains of the very first animals, struggle to confidently identify them as such, simply because they have no clear idea of what these animals were supposed to look like. Such uncertainties have shrouded the earliest history of animals in mystery.

Currently, the best candidates for the oldest possible animal fossils are members of the diverse and largely soft-bodied Ediacaran macrobiota, found in rocks dated to ~580–541 million years ago [11]. However, many of these organisms have proven difficult to interpret, with little agreement as to whether iconic taxa such as *Dickinsonia*, *Fractofusus*, or *Spriggina* (Figure 1) are animals, fungi or something else altogether [11]. Alternative lines of fossil evidence for early metazoans fare little better. Embryos from the Doushantuo Formation of China (~580 million years ago) considered to be of putative animal origin [12], are now widely interpreted to record developmental stages of organisms that are not animals [13]. An increasingly diverse suite of trace fossils, extending back to ~565 million years ago [14], indicates that motile animals were present during the late Ediacaran, but tells us little about their phylogenetic affinities: were the traces formed by long extinct ancestors of modern lineages (i.e. members of the stem animal tree) or early members of modern crown groups? Older records of metazoans from the early Ediacaran and the Cryogenian are even more equivocal, and are limited to recently reported 600 million year old fossils of putative sponge-like organisms [15] and possible demosponge biomarkers [16] from strata dated to 715–635 million years in age.

Zuckerkandl and Pauling's [8] suggestion that a 'molecular clock' could be utilised to date the tree of life requires that the clock is 'calibrated'; that is, that the rate at which genomes accumulate mutations is estimated in some way. For forty years after that initial proposal, much attention was devoted to deriving timescales of life that used minimal fossil calibrations to avoid introducing errors relating to uncertainties within the fossil record. Such studies found a general lack of agreement between fossil- and molecular-based timescales. However, in 2004 Dan

Graur and William Martin published a “sanity check”, in which they compared the molecular clock practices of the previous two decades to the hardly scientific practice of “reading the entrails of chickens” [17]. This way, they relegated two decades of molecular timescales built using inaccurate ’derivative‘ calibrations (i.e. calibrations based on the results of previous molecular clock studies that were interpreted as error free [17]), to the dusty folder of papers marked ‘historical interest only’. At the same time, Bayesian methods and software were revolutionising evolutionary research, permitting implementation of much more realistic molecular clock models [18], and perhaps most importantly, enabling calibration of molecular clocks using probability distributions to account for multiple fossil calibrations and their associated levels of uncertainty [1-4]. These novel approaches finally allowed the effective integration of molecular and fossil data when estimating divergence times, and eventually led to the emergence of consensus on the timing of the origin of animals. Indeed, most recent studies [1-4] broadly agree that the Cambrian explosion (the great animal diversification event that happened ~520 million years ago) should principally be seen as the time when most modern animal lineages (the phyla and classes) radiated. Similarly, they agree that the last common metazoan ancestor was Neoproterozoic, and specifically pre–Ediacaran, in age, most likely being younger than 800 million years [e.g. 1-4]. Surprisingly however, the ~200 million year interval between the hypothesised origin of animals and their Cambrian radiation has received relatively little palaeontological attention, with molecular-divergence times and the fossil record being significantly at odds for this period.

The results of dos Reis and colleagues [1] now demonstrate how difficult it is to precisely date evolutionary events close to the root of the animal tree. The authors used a large, genomic-scale dataset including 71 species sampled across all animal lineages. They compared four molecular clock calibration strategies spanning a range of interpretations of the existing fossil record, and also assessed the uncertainty introduced by different data partitioning strategies and variations in the rate of gene mutations among lineages [1]. While some of their results could probably be predicted (for example, increasing the number of data partitions and thus the numbers of parameters to be estimated during the analysis decreased the precision of the clock), others are illuminating. In particular, they showed that divergence times across the upper Cryogenian and Ediacaran are entirely driven by the way the pre-Cambrian fossil evidence is interpreted. This observation suggests that there is not enough signal left in genomic data to derive precise divergence times so deep in animal history without imposing well-defined fossil constraints to the analyses. This leads to three profound implications. First, the precision of molecular divergence times for early animal history relies on the quality of the

fossil evidence used to calibrate the clock; second, to improve the precision of these divergence times it is imperative to improve our knowledge of the earliest animal fossil record; third, if the molecular dataset of dos Reis *et al.* [1] is representative of genomic-scale datasets in general (which is most likely the case), 'fossil-free' molecular divergence times (relative divergence times), which were introduced to avoid the known problems of the fossil record and are based exclusively on the information in molecular sequence data [19], are meaningless in deep time.

This imprecision of the molecular clock deep in the history of life is frustrating. While the clock provided hope that divergence times for lineages could be dated in the absence of fossil information, it is now clear that the only way to increase its precision is to improve our knowledge of the fossil record itself, via the discovery of new fossils, resolving the affinities of existing ones, and accurately dating fossil occurrences. With genomic data now available [1] our focus should return to palaeontology, and particularly to the investigation of the early and middle Neoproterozoic. It is evident that in isolation, neither fossils nor molecular data can derive the precise and accurate timescale of life so essential to our efforts to robustly test proposed correlations between the history of life and that of planet Earth.

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Figure 1. Enigmatic Ediacaran macrofossils.

Some of the more enigmatic members of the Ediacaran macrobiota; competing hypotheses for their biological affinities are summarised in [10]. (A) *Haootia quadriformis*, a possible cnidarian from Newfoundland, Canada [20] (Photo: D. McIlroy). (B) *Kimberella quadrata* from South Australia, SAM P48935, a possible mollusc. (C) *Dickinsonia costata*, SAM P49355. (D) *Thectardis avalonensis*, a triangular form from Newfoundland, interpreted by some as a possible sponge. (E) *Spriggina floundersi*, SAM P29803. (F) *Fractofusus misrai*, a rangeomorph taxon from Newfoundland, Canada. The frondose rangeomorphs have been suggested to belong to a variety of different phyla and kingdoms. Scale bars = 10 mm.